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Citrus Canker: The Pathogen and Its Impact

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History and Overview

Fig. 1. Citrus canker lesions on immature fruit stems and foliage of grapefruit.

Increasing international travel and trade have rendered U.S. borders more porous and dramatically increased the risk of introductions of invasive plant pests into agricultural crops (3). The current USDA system for protecting agricultural industries has been overwhelmed and has sometimes failed to intercept a number of introductions of exotic pests, including plant pathogens. Such introductions threaten crops and can hinder national and international agricultural markets and trade. Currently in Florida, one such invasive species is *Xanthomonas axonopodis* pv. *citri* (*Xac*), a bacterial plant pathogen that causes Asiatic citrus canker (Fig. 1). Citrus canker is an introduced plant disease, the eradication of which has received considerable press attention and legal challenges, has produced far-reaching political and socioeconomic impact in Florida, and has implications for national and international trade (4,6). *Xac*'s leaf, stem, and fruit-blemishing directly reduce fruit quality and quantity. *Xac*'s presence, if detected, triggers immediate quarantines of areas with outbreaks in Florida, disrupting movement of fresh fruit (6,84).

Citrus canker has a long history in Florida. The disease was first found around 1912, spread throughout the southeastern U.S. on imported seedlings from Japan, and was declared eradicated from Florida and the adjacent states in 1933 (13,58). Citrus canker was discovered again in Manatee County, Florida, south of Tampa Bay in 1986 and was declared eradicated by 1994 (74). Three years later the disease re-emerged in the same general area on the west coast of Florida where the 1980s outbreak had occurred. In the meantime, a new and separate infestation of citrus canker was discovered in urban Miami in 1995, with an estimated introduction some time in 1992 or 1993 (25,68,69) (Fig. 2).

Fig. 2. Citrus canker outbreaks in south Florida peninsula. Red areas indicate location. Note the large red areas of Miami-Dade and Broward counties to the southeast and large area of Manatee County to the northwest.

When detected in Miami in 1995, the infected area was contained in approximately 36.3 km^2 (14 $mile^2$) of largely residential properties southwest of the Miami International Airport. In response to the 1995 detection of citrus canker, a cooperative state/federal citrus canker eradication program (CCEP) was established between the Florida Department of Agriculture and Consumer Services (FDACS), the Division of Plant Industry (DPI), and the USDA Animal and Plant Health Inspection Service (APHIS). Concurrently, citrus canker was rediscovered in commercial citrus in Manatee County on the west coast of Florida in June 1997, where a similar eradication effort is currently in progress. Subsequent outbreaks of citrus canker have also occurred in both residential and commercial citrus in Collier, Hendry, Hillsborough, Palm Beach, Martin, De Soto, Monroe, and Brevard counties of Florida whose origins are believed to be related predominantly to the inoculum reservoir in residential Dade and Broward counties. Despite extensive eradication efforts, which resulted in the removal or cutting back of over 1.56 million commercial trees and nearly 600,000 infected and exposed dooryard citrus trees statewide, the infected area has increased to 1701 km^2 (657 $mile^2$) as of March, 2002 (Fig. 3).

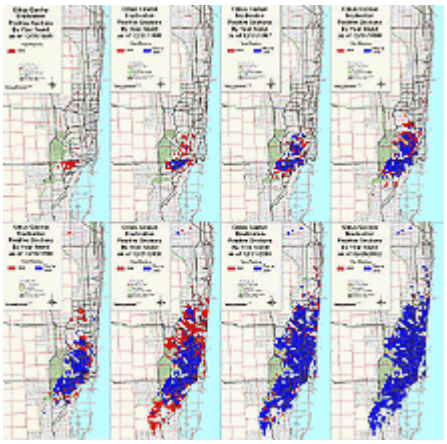

Fig. 3. Temporal progression of citrus canker infested sections in the Miami-Dade and Broward County area. Each square represents 1.0 mi^2 sections. Red squares represent new infested sections during that year. Blue squares are previous year(s) infestations.

The quarantine area is presently over 2590 km^2 (1000 $mile^2$) in urban southeast Florida in Miami-Dade and Broward counties; statewide the total quarantine areas encompass 3890 km^2 (1502 $mile^2$) (70). On the other hand, eradication has met with limited success in one area in southeast Florida (Sun City Center) to the point of actually lifting the quarantine. FDACS officials believe that more areas within some quarantine zones will reach the two-year mark with no canker detection (the point at which a quarantine may be lifted) in the next few months (70).

Citrus canker, is characterized by erumpent lesions on fruit, foliage, and young stems of susceptible cultivars of citrus (7,70,84) (Fig. 1). Unfortunately, most commercial citrus varieties grown in Florida are moderately to highly susceptible to the disease. When the disease is severe, defoliation (Fig. 4), dieback and fruit drop (Figs. 5 and 6) can occur and infected fruit that remain are less valuable or entirely unmarketable (34,48). During seasons when spring and summer rains are combined with wind speeds in excess of 18 mph (8m/s), damage from the disease can range from nominal to significant (71). The situation in Florida was exacerbated by the introduction of the Asian citrus leafminer, *Phyllocnistis citrella* Stainton, in 1993. Citrus canker has increased significantly as a consequence of the insect's feeding activities which create wounds that expose leaf mesophyll tissues to splashed inoculum, thus increasing the probability of infection by *Xac* (26).

Fig. 4. Back-lighted citrus tree showing defoliation due to severe canker infection.

Fig. 5. Hamlin sweet orange tree showing fruit drop due to citrus canker infection, Brazil.

Fig. 6. 'Pera' sweet orange tree showing fruit drop due to citrus canker infection, Brazil.

Genomic analysis of several pathogen isolates from the 1986-94 outbreak in comparison with the isolates found in the more recent Manatee County outbreak indicates the current outbreak is a result of incomplete eradication in 1994 (27). The 1986 Manatee strains are indistinguishable from the 1997 Manatee County strains. A less likely alternative is that the same genotype of the pathogen has been introduced twice to the same area. The majority of post-1997 outbreaks of

citrus canker in Florida are apparently the result of a single introduction of *Xac* in the Miami area (9,27). Thus, despite regulations concerning decontamination of personnel and equipment and strict prohibitions against citrus plant movement, human-assisted dispersal of the pathogen from Miami-Dade and Broward counties appears to have occurred several times. In early 2000, a third genetically identifiable strain of Asiatic citrus canker (Wellington strain) with an attenuated host range was identified in Palm Beach County on the east coast of Florida (79). Thus, there are at least three *Xac* genotypes known to have been introduced into Florida in the last two decades (Fig. 7).

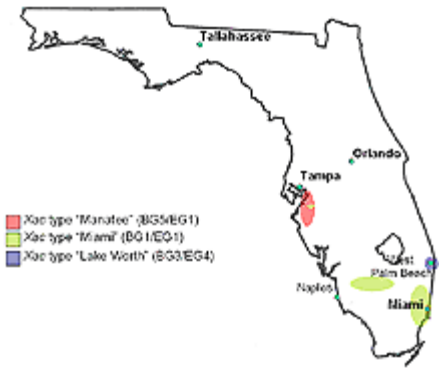

Fig. 7. Distribution of known citrus canker isolates in Florida. (Courtesy J. Cubero.)

In addition to tree debilitation and losses in quality and quantity of fruit, citrus canker results in devastating socioeconomic and political impacts because of the market standards for fresh fruit and perceptions of possible inoculum transmission on the fresh fruit product. (1,27). If *Xac* should become endemic in Florida, it will result in a severe curtailment of interstate and international commerce of fresh citrus fruit, which comprises approximately 20% of the state's $9 billion commercial citrus industry (61,62). Some yield reductions can be expected in the varieties grown for processing also. In addition, some cultivars and highly susceptible citrus species, in particular grapefruit (*Citrus paradisi*), will probably be impossible to grow profitably due to the requirements for multiple bactericidal sprays per year along with other canker management schemes to maintain yields and quality.

The Florida citrus industry is concentrated predominantly in the southern half of the state in close proximity to rapidly expanding urban population centers. Because the outbreaks originated in urban areas, FDACS and USDA response to citrus canker affects not just the citrus industry, but also hundreds of thousands of urban homeowners who have citrus trees as ornamentals and for dooryard fruit production and have had or will have their trees destroyed. This represents a situation that is becoming all the more common in the U.S., one in which agricultural emergencies originate or spill over into private non-agricultural environments and require decisions based on the overall good of society. Some of these decisions may favor industry; others may not.

Citrus Canker Strain Diversity, Identification, Diagnosis and Characterization

Strain types. There are distinct types of citrus canker disease caused by various pathovars and variants of the bacterium, *X. axonopodis* (syns. *X. campestris, X. citri*). Because symptoms are generally similar, separation of these types from each other is based on host range, cultural and physiological characteristics, bacteriophage sensitivity (7), serology (2), plasmid fingerprints (65), DNA-DNA homology (14), and by various RFLP and PCR (polymerase chain reaction) analyses (9,40,41,42,43,85). The latter DNA-based assays demonstrate that these strain types are genetically as well as pathologically unique.

The Asiatic type of canker (Canker A), caused by a group of strains originally found in Asia, is by far the most widespread (Fig. 8) and severe form of the disease. This is the group of *X. axonopodis* pv. *citri* strains that causes the disease most referred to as Asiatic citrus canker (Fig. 9). Minor genetic variation of citrus canker strains has been detected in the A strains in Florida and other citrus growing regions of the world, which may be exploited to identify their origin when introduced into new locations.

Cancrosis B, caused by a group of *X. axonopodis* pv. *aurantifolii* strains originally found in S. America is a disease of lemons, Mexican lime, sour orange, and pummelo. Strains of the Cancrosis B group can be easily differentiated from strains of the Canker A group (Fig. 10).

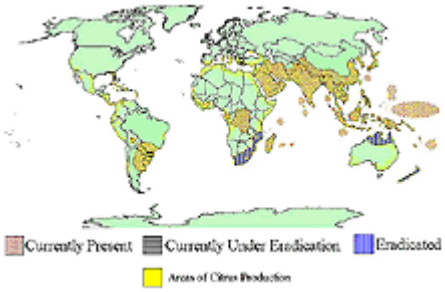

Fig. 8. Worldwide distribution of citrus canker and where eradication is practiced.

Fig. 9. Red grapefruit leaf with citrus canker lesions surrounded by chlorotic halos.

Fig. 10. Lemon fruits infected with the B-strain of citrus canker from Argentina.

Cancrosis C, also caused by strains within *X. axonopodis* pv. *aurantifolii,* was isolated from Mexican lime in São Paulo State, Brazil. The only other known host for this bacterium is sour orange. Strains of this group cannot be easily differentiated from strains of Cancrosis B group.

A fourth group of strains, known as A*, was discovered in Oman, Saudi Arabia, Iran, and India. This group is limited in host range to Mexican lime and appears to be distinct from the common A strains (85). An atypical form of A strain has been described from Reunion and surrounding islands in the Indian Ocean. It has high levels of resistance to a number of antibiotics. A single strain identified as Canker D, was reported in 1981 on Mexican lime in Mexico, but its identification remains controversial (60).

Identification of Strain Types for Diagnosis. Pathogenicity of unknown isolates on leaves of different citrus hosts including grapefruit, Mexican lime, sour orange, and various types of lemons is an essential determination in diagnostic programs for regulation of citrus canker diseases (70). Due to the relatively slow rate of symptom development, definition of the host range of unknown isolates in comparison with a collection of strains may require several weeks. Simultaneously, several other diagnostic tests are

conducted to verify the type of canker strain, including monoclonal antibodies, MIDI fatty-acid profiling, and genetic procedures using PCR probes (70).

PCR methods have been developed for rapid and accurate identification of the bacterium isolated in culture and from extracts of lesion on leaves and fruits (10,42). The primers used for citrus canker diagnosis are based on the plasmid containing the *pthA* gene, the primary virulence element in all citrus canker strains (43,87). Primers based on the *pthA* gene are available for detection of all canker strains in Florida and elsewhere (9). However, A-strain specific monoclonal antibodies failed to identify the A strain variant Aw (Wellington strain), recently discovered in Palm Beach County, Florida, and capable of inducing canker symptoms on a restricted range of citrus hosts including Mexican lime and alemow (*C. macrophylla)* (80).

A second approach that produced universal primers for *Xac* detection and identification is derived from specific sequences in the chromosome of the bacterium. PCR primers based on sequence variation in the intergenic spacer (ITS) regions of 16S and 23S ribosomal DNAs were designed specifically to identify the A strains and their variants. This set of primers readily differentiates all A strains from the B and C strains of *X. axonopodis* pv. *aurantifolia*.

Characterization of Strain Genotype. Rep-PCR with BOX and ERIC primers have also been used to not only separate canker strain types but also to differentiate strains within the same pathotype (9,59). This methodology can be applied to evaluate the diversity of *Xanthomonas* strains causing citrus canker in Florida and to relate these strains with a worldwide collection to establish their possible geographic origin (9). Rep-PCR supports the inclusion of Aw strains among A strains and reveals diagnostic genotype differences among A strains in different geographic areas of Florida. This genotyping also supports the idea that isolates of the original outbreak in Manatee County in 1986, an infestation that was supposedly eradicated in 1994, are the same genotype that reemerged in 1997 (9,70). The rep-PCR fingerprint for the MA strain from Manatee County, Florida, also matches that of strains from China and Malaysia. A second genotype from the outbreak Miami (MI) matches strains from several geographic areas of the world including Southeast Asia and South America. The MI genotype is detected in several locations in south and central Florida and thus traces of movement of plant material and other human activities back to the Miami metropolitan area. The close relationship of Aw with the A* strain suggests a common origin of these strains in Southwest Asia. Overall, genotyping with rep-PCR validates the existence of at least 3 separate A strain introductions in Florida over the last 20 years.

Symptoms and Infection Process

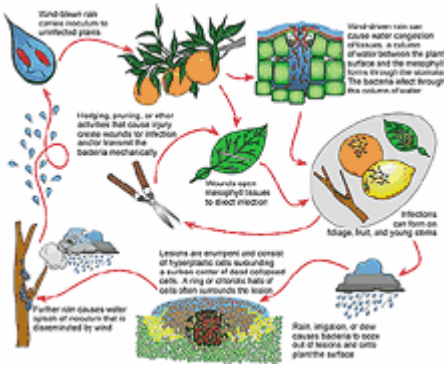

Fig. 11. Citrus canker disease cycle.

Disease Cycle. The bacterium propagates in lesions in leaves, stems, and fruit. When there is free moisture on the lesions, bacteria ooze out and can be dispersed (Fig. 11) to new growth and other plants. Rainwater collected from foliage with lesions contains between 105 to 108 cfu/ml (16,77). Wind-driven rain is the main natural dispersal agent, and wind speeds ≥18 mph (8 m/s) aid in the penetration of bacteria through the stomatal pores (Figs. 12 and 13) (22,37) or wounds made by thorns (Fig. 14), insects such as the Asian leafminer (Fig. 15), and blowing sand (81). The serpentine mines under the leaf cuticle (Fig. 16) caused by the larvae (Fig. 17) of the Asian citrus leafminer, a pest first detected in 1993 in Florida (44), provide ample wounding on new growth to greatly amplify citrus canker infection (8,67,72,73) (Fig. 18). Water congestion of leaf tissues can be seen following

rainstorms with wind. Citrus foliage can hold 7 microliters/cm^2 of leaf area (17). Studies of inoculum associated with water congestion have demonstrated how as few as 1 to 2 bacterial cells forced through stomatal openings can lead to infection and lesion formation (22,37). Wind blown inoculum was detected up to 32 meters from infected trees in Argentina (76). However, in Florida, evidence for much longer dispersals (up to 7 miles) associated with meteorological events such as severe rainstorms and tropical storms has been presented (24,27). Pruning causes severe wounding and can be a site for infection (Fig. 19).

Fig. 12. SEM of stomata on grapefruit leaf with *Xac* bacteria entering stomatal chamber. (Courtesy J. Cubero.)

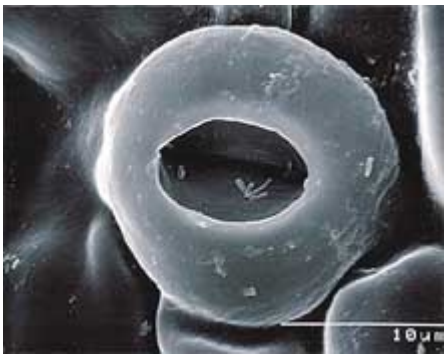

Fig. 13. SEM of stomata on grapefruit leaf with *Xac* bacteria in stomatal chamber. (Courtesy J. Cubero.)

Fig. 14. Lemon leaf with thorn scratches infected with *Xac*.

Fig. 15. Citrus leaf demonstrating Asian leafminer gallery (right) and second gallery in which citrus canker was introduced and has infected the exposed mesophyll (left).

Fig. 16. Citrus canker/Asian leafminer interaction demonstrating infections resulting from *Xac* bacteria dragged by larvae through gallery while feeding.

Fig. 17. Adult moth and larvae of Asian leafminer in feeding gallery.

Fig. 18. Multiple young citrus canker lesions erupting through leafminer feeding gallery.

Fig. 19. Pruning (hedging) of a citrus grove: An excellent way to cause tremendous wounds and spread citrus canker.

Multiplication of bacteria occurs mostly while the lesions are still expanding, and numbers of bacteria produced per lesion is related to general host susceptibility (Fig. 20). The bacterium remains alive in the margins of the lesions in leaves and fruit until they fall and begin to decompose. Bacteria also survive in lesions on woody branches up to a few years of age (Fig. 21). Bacteria that ooze onto plant surfaces die upon exposure to drying. Death of bacteria is accelerated by exposure to direct sunlight. Exposed bacteria survive only a few days in soil and a few months in plant refuse that is incorporated into soil. On the other hand, the bacteria can survive for years in infected tissues that have been kept dry and free of soil (17).

All aboveground tissues of citrus are susceptible to *Xac* when they are young, and at maximum susceptibility during the last half of the expansion phase of growth (22,77). Bacterial cells ooze from existing lesions during wet weather to provide inoculum for further disease development (84). Like many other bacterial diseases, the pathogen enters host plant tissues through stomates (22,37) and wounds (81). The earliest symptoms on leaves appear as tiny, slightly raised blister-like lesions around 7 days after inoculation under optimum conditions. Optimum temperature for infection falls between 20 and 30°C (48). Under less than optimum infection and incubation conditions, symptoms may take 60 days or more to appear (22,58). As the lesions age, they first turn light tan, then tan-to-brown, and a water-soaked margin appears, often surrounded by a chlorotic halo (Fig. 22). The water-soaked margin may disappear as lesions age, and is not as prominent on resistant cultivars (Fig. 23). The center of the lesion becomes raised and spongy or corky (Fig. 24). These raised lesions from stomatal infection are typically visible on both sides of a leaf. Eventually, the centers of leaf lesions become crater-like and may fall out, creating a shot-hole effect. Defoliation becomes a problem as the disease intensifies on a plant (21,28,32).

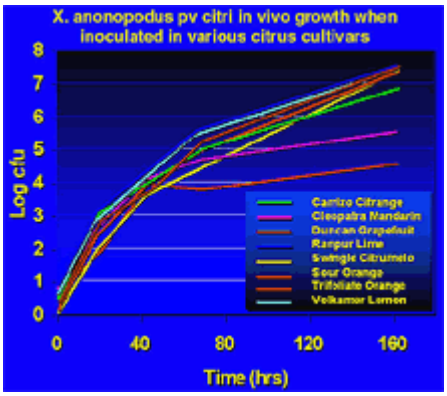

Fig. 20. *In vivo* growth of citrus canker in various citrus species and citrus relatives.

Fig. 21. Stem lesion on woody citrus branch ca. 2.0 cm diameter.

Fig. 22. Back-lighted grapefruit leaf demonstrating citrus canker lesions and chlorotic halos.

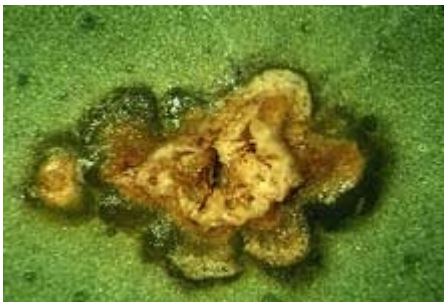

Fig. 23. Close up of citrus canker lesion demonstrating crater-like appearance and water soaking on margins.

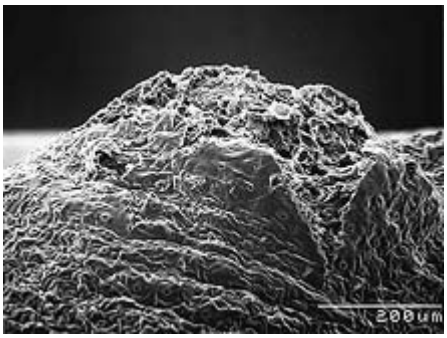

Fig. 24. SEM of erumpent citrus canker lesion. (Courtesy J. Cubero.)

On twigs and fruit, citrus canker symptoms are similar: raised corky lesions surrounded by an oily or water-soaked margin (83). No chlorosis surrounds twig lesions (Fig. 25), but may be present on fruit lesions (Fig. 26). Twig lesions on angular young shoots perpetuate *Xac* inoculum in areas where citrus canker is endemic. Twig dieback, fruit blemishes (Fig. 27), and early fruit drop (Figs. 5 and 6) are major economic impacts of the disease in advanced stages. If twigs are not killed back by girdling infections, the lesions can persist for many years, causing raised corky patches in the otherwise smooth bark (Fig. 21).

Fig. 25. Citrus canker lesion on sweet orange stem.

Fig. 26. Large citrus canker lesions on immature fruit with chlorotic halos.

Fig. 27. Mature grapefruit fruit with canker lesions making fruit unmarketable.

As a general rule, *Xac* is capable of naturally infecting green citrus tissues most readily while they are in the last half of expansion phases of growth. Once leaves, twigs, and fruit reach mature size, a thickened cuticle forms, they begin to harden off physiologically, and become more resistant to infection (78). Very young tissues with immature, unopened stomata, are also resistant to natural stomatal infection, but may suffer wound infections. Mature aboveground tissues can also be infected through wounds. Since young growth provides the bulk of the susceptible tissues, vigorously growing trees are most threatened by *Xac*. A well-managed citrus tree in Florida will undergo three to five growth flushes every growing season, each accompanied by a period of enhanced susceptibility.

Similar to other bacterial diseases, wind-driven rain is the primary short- to medium-distance dispersal mechanism for citrus canker (28,31). Long-distance spread normally occurs by human movement of diseased or exposed citrus plant material or by use of equipment contaminated by exposure to diseased citrus. Strong circumstantial evidence points to occasional medium to long-distance transport by unusual storm events such as tornadoes and tropical storms (25,26).

Xac easily persists from one growing season to the next in old lesions, especially lesions formed late in the growing season. *Xac* can remain viable as long as host cells in the vicinity of the lesion are alive, although the bacterial titer will drop considerably (82,84,85). Stem lesions can harbor viable bacteria for several years. Viable bacteria were recently isolated from stem lesions on 5- to 7-year-old trunk of Mexican lime infected with the Wellington strain of the canker pathogen in Florida (X. Sun, *unpublished data*).

Attempts to detect surviving bacteria on various inanimate surfaces such as metal, plastics, cloth, and processed wood in both shade and sun indicate the

inoculum dies within 24 to 72 hours (39). *Xac* may persist for several weeks on non-host plant material under natural conditions (63). Longer persistence (up to 8 months) in the root zone of certain grasses under eradicated diseased trees has been reported in Japan (19,64) and Brazil (64), although no other studies have confirmed such findings. Once diseased or exposed fruit or leaves drop to the ground, the bacterial population declines to a non-detectable level in 1 to 2 months because of antagonism and competition with saprophytic microorganisms (40,52). The abundant extracellular polysaccharide slime layer that encapsulates the bacterial cells (18) aids inoculum survival.

Citrus canker bacteria, *Xac*, can frequently be detected in soil, citrus roots, and from 17 various weed species collected in citrus orchards before new shoots develop in the spring (20,63). *Xac* survives in close association with roots of weed species and does not decline during the winter months (19). Experiments with artificially infected soils indicated that *Xac* survival in the soil is limited to approximately 1 week (15,51,57); however, more recent studies with buried citrus leaves with lesions have demonstrated that *Xac* can survive for 2 to 3 months before declining to nondetectable levels (36,40). However, there is to date no direct evidence that *Xac* surviving in low numbers on weed hosts or in the soil can serve as sources of inoculum for epidemic development (20).

Fig. 28. Fully sized but non-colored grapefruit fruit with 6-mo., 3-mo., and 1-mo.-old lesions, respectively from left to right.

When disease is discovered in a particular location, regulatory action is based in part on how long the disease has been active there. The number of infected plants offers one clue. Infection age on a single plant offers another. Because the infection process and syndrome development on this perennial woody plant are well understood and must occur naturally on tissues of a certain age, it is possible to determine the approximate age of the lesions. If symptoms are detected on leaves of the latest flush only (the tissues most likely to be infected), the disease was initiated only a few weeks or months before. On a well-nourished, susceptible host plant under environmental conditions conducive for disease, the expansion rate of leaf lesions is estimated at about 1 mm per month for the first 6 to 8 months. Leaf lesion expansion slows and stops at around this age. The susceptible period of fruit enlargement is typically 90 to 120 days after fruit set (38), so lesions on enlarging fruit can be dated based on bloom timing (Fig. 28). Twig lesions are generally initiated only after leaves and possibly fruit have gone through one or more infection cycles. Appearance of fruit and twig lesions also assumes that in most cases a certain prior inoculum level must have been reached on leaves to further advance the disease. Because each growth flush leaves a distinctive node on the twig, the determination of twig lesion age is a matter of dating backwards from the number of flushes indicated. Older lesions on bigger stems with brown bark can be dated by dendrochronological methods, keeping in mind that each growth ring records a flush and not an annual ring. One difficulty is determining the number of flushes that have occurred on that particular diseased plant within the preceding growing season(s). On trees managed for commercial production, lesion age determination is less of a challenge than on a residential tree with varying levels of typically less salubrious horticultural care.

Fig. 29. Red grapefruit fruit with massive coalescence of canker lesion resulting in large "pancake" lesion, and leaf with large single lesion from infection when leaf was immature.

Host Range. Among citrus cultivars and rootstocks, citrus canker is most severe on grapefruit (Fig. 29), some sweet oranges such as Hamlin, Pineapple, and Navel (Fig. 30); Mexican (Key) limes (Fig. 31) and lemons (Fig. 32), and trifoliate orange [*Poncirus trifoliata* (L.) Raf.] (Fig. 33) and their hybrids that are used for rootstocks (Table 1). These cultivars have proven very challenging or impossible to grow profitably in the presence of citrus canker in moist subtropical and tropical climates. All other commercial cultivars of citrus, though varying in susceptibility, are susceptible enough that they must be removed in an eradication effort when diseased or exposed. Civerolo (7) lists a number of plants in the Rutaceae other than *Citrus* and *Poncirus* that can serve as hosts of *Xac* under experimental conditions or heavy disease pressure in nature. These plants would not be expected to play any significant role in citrus canker epidemiology where the disease is endemic, but could serve as problematic inoculum reservoirs in an eradication or suppression program.

Fig. 30. 'Hamlin' oranges with citrus canker lesions resulting from infection early in fruit development.

Fig. 31. Mexican (Key) lime foliage with citrus canker lesions.

Fig. 32. Mature lemon fruit attached by citrus canker from Argentina.

Fig. 33. *Poncirus trifoliata* foliage (citrus relative used as rootstock) infected with citrus canker.

Table 1. Relative susceptibility/resistance to citrus canker of commercial citrus cultivars and species.

Rating	**Citrus cultivars**
Highly resistant	Calamondin (*C. mitus*); Kumquats (*Fortunella* spp.)
Resistant	Mandarins (*C. reticulata*) -- Ponkan, Satsuma, Tankan, Satsuma, Cleopatra, Sunki, Sun Chu Sha
Less susceptible	Tangerines, Tangors, Tangelos (*C. reticulata* hybrids); Cravo, Dancy, Emperor, Fallglo Fairchild, Fremont, Clementina, Kara, King Lee, Murcott , Nova, Minneola, Osceola, Ortanique, Page, Robinson, Sunburst, Temple, Umatilla, Willowleaf (all selections); Sweet oranges (*C. sinenesis*) -- Berna, Cadenera, Coco, Folha Murcha, IAPAR 73, Jaffa, Moro, Lima, Midsweet, Sunstar, Gardner, Natal, Navelina, Pera, Ruby Blood, Sanguinello, Salustiana, Shamouti, Temprana and Valencia; Sour oranges (*C. aurantium*)
Susceptible	Sweet oranges - Hamlin, Marrs, Navels (all selections), Parson Brown, Pineapple, Piralima, Ruby, Seleta Vermelha (Earlygold), Tarocco, Westin; Tangerines, Tangelos -- Clementine, Orlando, Natsudaidai, Pummelo (*C. grandis*); Limes (*C. latifolia*) -- Tahiti lime, Palestine sweet lime; Trifoliate orange *(Poncirus trifoliata*); Citranges/Citrumelos (*P. trifoliata* hybrids)
Highly susceptible	Grapefruit (*C. paradisi*); Mexican/Key lime (*C. aurantiifolia*); Lemons (*C. limon*); and Pointed leaf Hystrix (*C. hystrix*)

(References for above are 17,23,33,53,54,88).

One aggravating factor that greatly favors dissemination of the disease is the Asian citrus leafminer, whose feeding on the epidermal cell layer forms galleries beneath the foliar cuticle. Cracks in the cuticle result in direct exposure of the mesophyll tissues to infection by *Xac* and massive lesions can result (Fig. 34). The combination of *Xac* and the leafminer can lead to significant field infection even on highly resistant cultivars and species of citrus such as calamondin and kumquat (T. R. Gottwald, *unpublished*). Although the relative susceptibility rankings remain valid in spite of the introduction of the Asian citrus leafminer into Florida, all cultivars of citrus are now much more vulnerable due to the wounding caused by larval feeding. Cultivars once thought resistant enough to be easily grown in the presence of citrus canker frequently have been found diseased in residential plantings (Fig. 35). As a rule, proximity to more susceptible citrus cultivars greatly increases the chances of infection on more resistant cultivars.

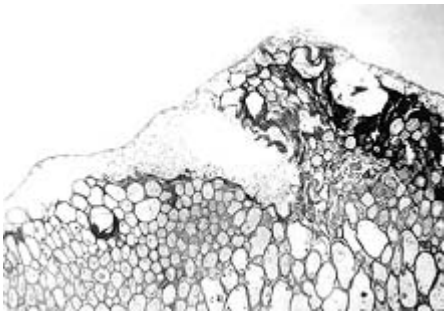

Fig. 34. Cross section of Asian citrus leafminer feeding gallery. Note *Xac* bacteria in gallery. (Courtesy D. S. Achor.)

Fig. 35. Lemon tree from Miami dooryard with severe Asian citrus leaf miner/citrus canker interaction demonstrating inoculum build up potential.

In an eradication program, it is important to identify all prospective hosts of the target pest or pathogen. Recently, Kalita (46) reported that goat weed (*Ageratum conyzoides* L.) in India serves as a host of *Xac*. This plant is common in citrus orchards in India, and is reported from isolated locations in Florida. This represents the only report of a non-Rutaceous natural host of *Xac*. Pathogenicity tests of *Xac* on *Ageratum conyzoides* in Brazil were negative (Rui Leite, *personal communication*), suggesting that the apparent infection of this non-Rutaceous plant may have been hypersensitivity resulting from extremely high inoculum challenges, and not true susceptibility (Table 1).

Epidemiology

The majority of epidemiology studies on citrus canker have concentrated on local disease increase and spread of *Xac* within citrus nurseries and commercial plantations. In citrus nurseries dissemination is primarily by splash dispersal (32,71). The result is the development of numerous secondary foci that eventually coalesce in larger, irregularly shaped areas of disease, which makes the description and quantification of disease gradients difficult. Slopes of disease gradients associated with citrus canker in nurseries fluctuate over time because of disease-induced defoliation on severely diseased nursery plants and subsequent infection of newly emerging foliage (32). Highly significant aggregation of citrus canker-infected trees was associated with splash dispersal, which decreased as the secondary foci coalesced (32). A slightly higher within-nursery row than across-nursery row aggregation indicating spread between plants is slightly more likely when they are closer together. Aggregation was demonstrable in all nurseries studied throughout the epidemics, and was not greatly influenced by direction (32).

For citrus canker epidemics in citrus orchards in Argentina, slopes of disease gradients were seen to fluctuate. This fluctuation was due to cycles of disease-induced defoliation, re-foliation, increased disease on that new foliage, followed by defoliation again. However, unlike citrus nurseries, disease gradients were directional and were related to windblown rain. Gradients were shallowest (most extensive) downwind and steepest (least extensive) upwind from the foci of infection (28). Disease progress was also significantly greater in the downwind direction. Aggregation of diseased trees was also indicated throughout the epidemics (28). In an earlier study, linearized disease gradients became steeper through time indicating a more rapid increase in disease near the focus of infection (11).

The same data from Argentina for orchards and nurseries was used to examine the spread of citrus canker through time by spatial autocorrelation and spatio-temporal (ST) autocorrelation methods (29). These methods in

combination provide the ability to examine the evolution of an epidemic in both space and time simultaneously and led to the description of ST transfer functions in the form of a mixed ST autoregressive integrated moving average (STARIMA) models (29). The models demonstrated that changes in the relationships of diseased trees over distance closely followed the changes in the associated disease progress. That is, as disease progressed more rapidly, the disease moved over longer distances. Although nursery and orchard results differed, both demonstrated disease relationships that persisted over distance and through time.

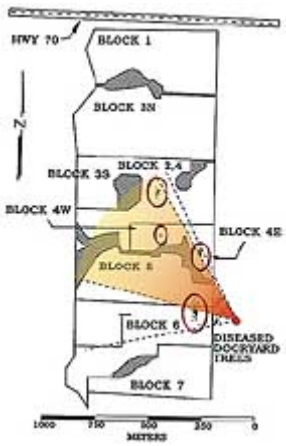

Fig. 36. Diagram of 5-year-old citrus planting that became infected with citrus canker from adjacent dooryard citrus tree. Note four newly established foci resulting from probable inoculum dispersal.

Because citrus canker is an exotic disease and under eradication in Florida, the opportunities to examine the epidemiology of the disease in the U.S. have been infrequent and predominantly involved a rapid assessment of a disease epidemic during the short interval between discovery and eradication. One such notable study involved citrus canker in a commercial orchard in south Florida in 1990 (24). The focus of infection was determined to be three, 8- to 10-year-old lemon trees in a rural home site that predated the planting of a large commercial orange orchard immediately to the west. An August 1989 rainstorm with associated high winds resulted in dissemination of inoculum and the establishment of four foci of infection in the orange orchard that ranged from 230 to 810 m (755 to 2657 ft) from the infected home site source trees (Fig. 36). This was the first documented spread of citrus canker over longer distances associated with rainstorms (24).

Regional analyses of disease increase and spread have rarely been undertaken, and regional studies of disease spread in urban environments do not exist. However, the largest outbreak of citrus canker in the U.S. occurred in urban Dade and Broward counties (Metropolitan Miami and Fort Lauderdale) of Florida. When the outbreak was first discovered in the fall of 1995, the disease was delineated in a ca. 32 km^2 (14-mi^2) area south west of the Miami International airport. In January of 1996 a severe rainstorm with tornados passed through this infected area on a southwest-to-northeast track. By mid-summer of 1996, canker had spread 9.6 to 11.2 km (6 to 7 mi) to the northeast and encompassed a 223 km^2 (86 mi^2) area (25,26,27). Such storms are common to the Florida peninsula with frontal boundaries stretching diagonally across Florida with prevailing winds along the frontal boundaries that often pump moisture and winds to the northeast (Fig. 37). It is this meteorologically driven spread combined with occasional human movement that is believed to have resulted in the continual migration of citrus canker northward up the east coast of Florida's most dense residential area.

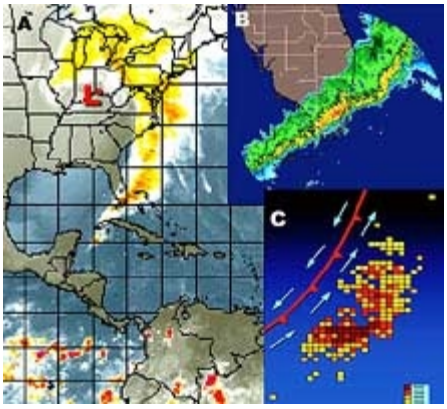

Fig. 37. (A) Map of eastern U.S. with barometric depression, L, and surrounding comma shaped weather pattern. (B) Doppler radar scan of same weather system as front passes through south Florida. (C) Diagram of frontal winds and rain and distribution pattern of one-square-mile sections infested with citrus canker.

Until recently, the scientific basis for the eradication effort of citrus canker in Florida was provided by previous data from Argentina indicating that canker bacteria can spread up to 32 m (105 ft) during rainstorms associated with wind (77). This was translated into regulatory policy that resulted in the location of diseased citrus trees by survey teams, and the removal and destruction of these trees and of "exposed trees" within a 38.1 m (125 ft) radius of a diseased tree (27). Brazil used a similar distance, 30 m (98 ft), to define exposed trees for removal (Fig. 38). However, despite the use of the "125-ft rule" by the CCEP, the disease continued to increase in southeast Florida urban areas and spread to numerous commercial citrus plantations across south Florida (25). Thus, questions arose concerning the appropriateness of the 38.1-m radius around diseased trees to identify exposed trees for eradication in an urban setting and resulted in a request by the CCEP for a study to examine the appropriateness and effectiveness of the rule. The main concerns were: (1) whether experimental data collected under Argentine meteorological conditions were applicable to the Florida situation; and (2) whether the Argentine data collected under orchard conditions were applicable to the urban situation where much of the eradication effort is ongoing in Florida. In response to these queries, a cooperative CCEP, ARS, and UF research effort was established in August 1998.

Fig. 38. Aerial view of citrus canker eradication methods deployed in Brazil. Circles represent 30-m removal of trees when disease incidence is < 0.05 whereas removal of

entire block (center rectangular area) is required when incidence > 0.05.

No prior information or studies exist on the spread of citrus canker in urban areas where dooryard citrus is the major source of inoculum. Therefore, a series of studies were conducted in five areas in urban Miami in an attempt to provide accurate information on the spread of *Xac* for the development of biologically sound and effective eradication/suppression procedures under urban conditions (30). To accomplish this, 18,769 trees in dooryards were surveyed, mapped using a global positioning system, and assayed for disease severity, age of infection, citrus cultivar, location of infection in tree, and canopy size. For each tree, the date the tree became infected was estimated and used to separate trees into contiguous 30-day categories. For each area studied, distance measurements between focal trees and newly infected trees were calculated for various temporal windows of 30, 60, 90, and 120 days in duration, corresponding to intervals of inspection survey (27,30). Distances between each newly diseased tree and all prior focal trees were calculated. For the first four 30-day periods among the five study areas, calculated maximum distances of spread ranged from 12 to 3474 m, indicating that bacterial spread was possible over a broad range of distance. Disease increased during the first two-thirds of the time studied then reached an asymptote due to dry conditions in the final one third of the duration of the study. Cross correlation analysis indicated that disease was optimally visualized by inspectors 107 days following rainstorms with wind (30).

Fig. 39. Map of a portion of residential Miami-Dade County, Florida. Blue dots depict examples of two individual citrus canker-infected trees located in two residential properties. Blue circles surrounding the blue dots indicate areas within 1900-ft radii of these two infected trees. Red dots indicated all known citrus canker-infected trees within the mapped area. Red lines depict the boundary defined by overlapping circles of 1900-ft radii that define the potential area exposed to citrus canker and within which all citrus canker-infected and non-infected trees are removed in an attempt to eradicate the disease.

The preliminary results of this study were examined by a group of scientists, regulators, and citrus producers familiar with the disease. They selected a distance of 579 m (1900 ft) as a radius that would encompass the majority of newly infected trees that can occur within a 30-day period resulting from a prior infection focus. The study and the resulting determination of the 579-m distance serve as the scientific basis of the eradication policy utilized in Florida at this time (30) (Fig. 39).

The citrus canker data were also examined by modified Ripley's methods to study changes in the regional spatial point pattern (SPP) of citrus canker through time in an urban setting (66,86). In general, aggregation (expressed by the effective Range of Spatial Dependency) increased concomitantly with disease incidence across all study sites and approached a maximum during the first few temporal periods, then tended to plateau indicating a general and sustained level of aggregation had been achieved. The data for the large urban study sites were also subjected to a spatio-temporal analyses over 25 30-day periods via spatio-temporal semivariogram analysis followed by kriging (30). When used in combination with Ripley's modified analyses, these methods demonstrated rapid increases in range of spatial dependency and range of spatio-temporal dependency for all study sites. This corresponded to rapid spread of disease across the regions studied in response to rainstorms with wind followed by a "filling in" of disease on remaining non-infected susceptible trees through time by less intense rain events (30).

The host population in urban areas is exceedingly non-uniform and thus host tree susceptibility is dynamic. Unlike a commercial citrus orchard, urban trees are not of uniform age, cultivar, or horticultural care. Therefore, the number and duration of new flushes of foliage continually changes over time and is dependent on cultivar, age, fertilization and general health of a tree. These horticultural factors varied widely and were continually in flux within the urban areas studied. A stochastic quadratization technique was used in large urban areas and demonstrated that disease incidence and disease severity were not greatly affected by urban host density but were positively correlated to host susceptibility within 0.25 km^2 quadrat areas (30).

Leafminer Interaction. Prior to 1994, the citrus leafminer had been restricted in distribution primarily to Southeast Asia. After the mid 1990s, the leafminer spread to most of the major citrus producing areas of the world, arriving in Florida in 1993 (44) and was first reported in Brazil in 1996. In the Miami area, the interaction between Asian citrus leafminer infestation and citrus canker was immediately apparent. The leafminer infests young citrus flush including both leaves and young stems. Young fruit, especially grapefruit, are also occasionally attacked. Leafminer larvae form feeding galleries in the epidermal cell layer of young leaves and other tissues, lifting and eventually tearing the cuticle (1,35). The feeding activities of the leafminer facilitate *Xac* infections in two ways. First, the tearing of the cuticle opens the mesophyll of the leaf to direct bacterial infection when splash-dispersed or windblown-rain-dispersed bacteria come in contact with the leaf surface (Fig. 34). Second, the leafminer larvae may become contaminated with bacteria and transport them through the feeding galleries. This results in numerous mesophyll infections within the galleries (35) (Figs. 15 and 16). As these numerous leafminer-induced lesions expand, they rupture through the epidermis, and coalesce to form massive infections covering large areas of the leaf lamina (Fig. 18). This greatly increases the infected foliar area and generates many times the amount of inoculum compared to *Xac* infections where the leafminer is not present (35). Large amounts of inoculum exude from leafminer-induced lesions, which promotes spread of the bacteria by rain splash and accelerates the epidemic. On some trees, citrus canker infections are restricted entirely to leafminer wounds. However, there are no published data that the leafminer serves as a true vector of canker inoculum. A field study conducted in South Broward County in January 2002 indicated that approximately 60% of the diseased citrus leaves were associated with leafminer injury and that the number of citrus canker lesions increased significantly on the leafminer infested leaves compared to leaves infected through the stomates (X. Sun, *unpublished data*).

Wounding by the Asian citrus leafminer accelerates the spread of citrus canker in Florida. Epidemiological studies of citrus canker conducted in the Western Hemisphere prior to the appearance of the leafminer (11,12,24,29) underestimate current disease increase and spread, since both the incidence of wounds that serve as infection courts and the amount of inoculum produced in a lesion have increased dramatically (5). The citrus canker epidemic was greatly exacerbated in Brazil, especially in São Paulo State where the number of disease foci increased from 25 in 1995 to 4,180 in 1999. In 1999, the disease was detected in 299,856 trees. As a consequence, 1,737,545 trees were eradicated. This rapid temporal increase of diseased trees occurred concomitantly with a change in the spatial pattern of the epidemic. Strongly aggregated patterns, typical of citrus canker from 1957 to 1995, gave way to less aggregated and even "at random" patterns and presence of satellite foci far away from main foci became very common (5). This change in the spatial pattern of diseased trees is thought to be caused by the leafminer, despite the fact that the insect is not a vector of the pathogen. Leafminer wounds are very susceptible to infection by bacterial aerosols formed during turbulent weather. Leafminer wounds are very different from natural wounds and are characterized by: (i) a delay in the plant healing reaction (one day for a wound caused by wind, thorns, or pruning versus 10 to 14 days for wounds caused by the miner); and (ii) lower inoculum doses to cause disease (1/100 to 1/1000 of the dose required for infection through natural openings).

Based on the analysis of 203 disease incidence maps of infected orchards just prior to eradication, it was proposed that the presence of the leafminer changed the dispersal function of the disease by flattening the dispersal gradient over a greater distance. As a consequence of this study, a new law was enacted in the state of São Paulo in July 1999, whereby groves with incidences higher than 0.5% are completely eradicated and those with incidences at or below 0.5% have all diseased and exposed trees within a radius of 30 m destroyed (5).

Management for Prevention and Control of Citrus Canker

Countries where citrus canker does not occur or has been eradicated rely on quarantine measures to prevent the introduction and establishment of *Xac*. Historically, outbreaks of citrus canker in the U.S., South Africa, New Zealand, and Australia are thought to have originated in Asian countries, although such outbreaks could originate from any country outside Asia with citrus canker. In newly-established outbreaks, programs that started immediately were successful in eradication of citrus canker, but only after large numbers of trees were destroyed. Although many citrus-producing countries prohibit the importation of plant material from citrus canker-endemic areas, outbreaks continuously occur in new areas of Florida, South America, and Australia. In some cases, eradication efforts have met with limited success in containing the spread of the disease in Florida, Brazil, Argentina, and Uruguay (45,48,70,75).

Integrated Management Programs. In regions where citrus canker is endemic, integrated control measures rely most heavily on the planting of resistant varieties of citrus. Since the industries in citrus canker-endemic areas coevolved with the disease, costs of disease management here are different from citrus production areas such as Florida that have developed over many decades in the absence of citrus canker. In Southeast Asia, where climatic conditions are most favorable for epidemics, the dominant cultivars grown are based on mandarins. Citrus canker has not been a serious problem until more susceptible sweet oranges were introduced into disease prone areas of Japan and China (48). In Brazil, eradication/control programs have been on-going since the 1950s to control the spread of *Xac* into the largest sweet orange production area in the world: São Paulo State. In contrast, nearby regions of Paraná State, Brazil, and Misiones and Corrientes, Argentina, have practiced an integrated program for effective prevention and control of citrus canker in sweet oranges (55). The strategies of the integrated program for citrus canker control are based on research carried out in the 1960s and early 1970s in Japan, and later in the 1970s in Argentina and 1980s in Brazil (47,50,55,56,78).

The most important feature of this program is the shift in planting from susceptible to field resistant citrus cultivars. Regulations in these regions not only address the requirement for more resistant cultivars, but also mandate production of *Xac*-free nursery trees and other means for exclusion of canker from orchards. Guidelines also specify management practices for citrus canker, and marketing of fresh fruit and nursery stock (55). Under these regulations, nurseries can only be located in areas free of citrus canker. In orchard production areas designated as citrus canker-free, regulations are designed to prevent or reduce the risk of citrus canker epidemics through the establishment of windbreaks, construction of fences to restrict the access to the orchard, and the use of preventive copper sprays. Fresh fruit for internal and export markets is subject to inspection protocols for freedom of citrus canker symptoms on fruit in orchards and sanitation treatments in the packinghouse.

Exclusion/Sanitation Procedures. Local or regional eradication may be practiced to establish and maintain areas free of citrus canker for the planting of new orchards with cultivars of low to moderate susceptibility. In an attempt to prohibit the introduction of the disease, many citrus-growing areas restrict the importation of citrus from areas or countries known to have canker. New orchards are established only in areas without history of citrus canker for at least one year after effective eradication is achieved (55). The choice of planting site takes into account not only horticultural and climatic factors for cultivation of citrus, but minimizes environmental conditions favorable for the introduction of *Xac* and development of citrus canker. Sites exposed to strong winds are avoided

due to the higher susceptibility of the citrus trees, particularly in the early stages of development (55). Compliance of orchard workers with measures for thorough disinfection of clothes, shoes, orchard machinery, and harvesting equipment including boxes is also essential to maintain exclusion of the pathogen (Fig. 40).

Fig. 40. Citrus canker decontamination station at edge of commercial citrus planting to decontaminate personnel, vehicles, and equipment and thereby inhibit inadvertent bacterial spread.

Compliance agreements have been issued statewide to citrus producers in Florida requiring decontamination of personnel and equipment to reduce the risk of spread of mechanical and inadvertent human spread of *Xac*. This focuses attention on the situations of greatest risk for *Xac* survival and transmission. To determine the possibility of survival and transmission of *Xac* on and from various surfaces, bacterial survival has been evaluated on various materials, including wood (representing crates, ladders, etc.), cotton cloth (clothing), cotton gloves, plastic (fruit crates), metal (vehicles, lawnmower blades, etc.), leather (gloves and shoes), bird feathers, and animal fur. Bacterial inoculum from macerated citrus canker lesions was applied to these various surfaces to be tested. These surfaces were exposed to ambient meteorological conditions, in sun or shade under outdoor conditions in Miami. Survival was significant up to 48 h under sun and 72 h under shade, depending on the weather conditions during the test (temperature, humidity, etc.). This confirms that when surfaces are dry, *Xac* dies, but before drying there is a considerable period of risk of bacterial transmission. In Miami, where diseased trees are chipped after removal from dooryards, the debris produced is laden with *Xac* detectable by air sampling in the vicinity (10 to 20 ft) of the machinery. Aerosol inoculum is also capable of causing infection of wetted foliage located in the zone of bacterial dispersal. This finding led to procedural changes for when and where chipping can be safely conducted to minimize risk of bacterial dissemination in the area of tree destruction.

Cultivar Selection. Production of pathogen-free budwood of citrus cultivars is the foundation of the integrated management program for production of citrus cultivars with field resistance to citrus canker (Table 1). Screening programs have been initiated throughout the world to evaluate the reaction of citrus cultivars to the disease under the local environmental conditions (49,54). Due to their high susceptibility, grapefruit, Mexican lime, several early-to-midseason sweet oranges (e.g., Navel, Hamlin) are not recommended for planting unless very intensive control programs are to be undertaken (48,55). Alternatively, screening programs have recommended selected mid- and late season sweet oranges, mandarin hybrids (tangerines, tangelos, tangors), and Tahiti lime that have an acceptable level of resistance to citrus canker. These cultivars may be susceptible in the young stages and require sprays for control of citrus leafminer to prevent damage to emerging leaf flushes that predisposes them to infection. Adult trees flush less frequently, reducing leafminer activity such that an acceptable level of resistance expression allows for effective disease management with the integrated program including windbreaks and chemical control (54).

Fig. 41. Windbreak (back) of Grevellia trees used to suppress windblown rain as a control measure to reduce citrus canker infections and spread in Brazil.

Cultural Practices. Cultural practices including windbreaks, and pruning or defoliation of diseased summer and autumn shoots, are recognized throughout the world as important measures for the management of citrus canker (50,55,78). Windbreaks are the most effective measure for the control of the disease on susceptible citrus cultivars (31,50,55) (Fig. 41). Windbreaks alone or in combination with copper sprays may reduce disease incidence on leaves and fruits to non-detectable levels on field resistant cultivars (55). Pruning and defoliation of diseased shoots in combination with copper sprays as a complete control has also been effective in light outbreaks. Pruning of the citrus trees is performed during the dry season, when the environmental conditions are less favorable for spread of the bacterium from pruned to adjacent non-infected trees. However, pruning is very labor intensive and therefore expensive.

Fig. 42. Typical airblast sprayer in citrus planting used to apply agrochemicals such as copper for control of citrus canker.

Chemical Control. Worldwide, citrus canker is managed with preventive sprays of copper-based bactericides (47,50,52,56,78) (Fig. 42). Such bactericides are used to reduce inoculum build up on new leaf flushes and to protect expanding fruit surfaces from infection. Effective suppression of the disease by copper sprays depends on several factors, such as the susceptibility of the citrus cultivar, environmental conditions, and adoption of other control measures (50,52,55,56,78). As a stand-alone measure, control of citrus canker with copper sprays on resistant or moderately resistant citrus cultivars may be achieved, whereas adequate control on susceptible or highly susceptible cultivars requires the implementation of several control measures (50,54,56,78).

The timing and number of copper sprays for effective control of citrus canker is not only highly dependent on the susceptibility of the citrus cultivar, but on the age of the citrus trees, environmental conditions, and the adoption of other control measures. In general, 3 to 5 copper sprays are necessary for effective control of citrus canker on citrus cultivars with intermediate levels of resistance (52), whereas, in years with weather that is highly conducive for epidemic development of citrus canker, up to 6 sprays may be recommended (56). This work was done in Brazil, which is a seasonally drier and a less tropical climate than Florida. Therefore, we would anticipate the need for an even greater number of sprays in Florida to achieve effective control of canker.

Social, Political, and Legal Ramifications of Regulatory Policy

Quarantines. One of the regulatory responses to citrus canker in the U.S. is the establishment of federal quarantine boundaries. Although the precise placement of such boundaries is a complex issue with both biological and political ramifications, in Florida they are usually located two or more miles beyond any known infestation (70). Within quarantine areas, movement of all citrus plant material is restricted. This affects both the citrus industry and homeowners with citrus trees. Commercial citrus nursery sales are prohibited. Commercial production must be handled in designated packinghouses where fruit is treated with disinfestants. Some processing plants and packinghouses refuse to accept fruit from quarantine areas. Market distribution of fresh fruit

from regulated areas is often restricted. Harvesting and transport equipment are required to undergo disinfestation. Commercial citrus plantings are required to have decontamination stations at farm gates, a precaution that has recently become a state-wide requirement, even outside of regulated areas. Replanting of citrus, in both commercial groves and residential areas that have undergone eradication efforts, is illegal (with few exceptions in commercial plantings) until canker has been declared eradicated and the area has been free of the disease for two years. In residential areas, people are informed that transporting fruit to neighbors and family is illegal. Even lawn and garden services are required to decontaminate any equipment moved between properties. Intensive media coverage and public relations expertise are employed to publicize these measures.

Eradication. Once *Xac* is introduced into an area, elimination of inoculum by removal and destruction of infected and exposed trees is the most accepted practice to contain the disease and stop further spread (75). Eradication programs have taken place in Florida previously during 1910 to 1933, and again in the 1980s and 1990s. Similar eradication programs aimed at citrus canker have been undertaken in Argentina, Uruguay, Brazil, Paraguay, Fiji Island and at least five times in Australia. Some of the programs have succeeded, others are ongoing, and others failed and were abandoned. To accomplish eradication, in Florida as elsewhere, trees are uprooted (Fig. 43) and burned (Fig. 44). In urban areas, trees are cut down and chipped (Fig. 45), and the refuse is disposed of in a landfill. Until recently, the scientific basis for the eradication effort was provided by previous data from Argentina indicating that canker bacteria can spread up to 32 m (105 ft) during rainstorms associated with wind (77). In the U.S., this was translated into regulatory policy that resulted in the location of diseased citrus trees by survey teams, and the removal and destruction of these trees and of exposed trees within a 38.1 m (125 ft) radius of a diseased tree (30,70). Brazil currently uses a similar distance, 30 m (98 ft), to define exposed trees for removal, but bases its use on disease incidence in commercial plantings. That is, if Brazilian plantings have 0.5% infection or less, all trees within 30 m of infected trees are removed, whereas if infection is greater than 0.5%, the entire block is removed (Fig. 38).

Fig. 43. Front end loader uprooting citrus canker infected trees in commercial grove in Martin County, Florida.

Fig. 44. Pile of uprooted diseased and exposed citrus trees being burned to eliminate citrus canker from commercial planting.

Fig. 45. Dooryard citrus tree being chipped by mechanical mulcher used to dispose of diseased and exposed citrus trees in residential areas.

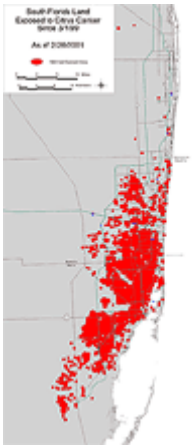

Fig. 46. Results of the 1900-ft regulation for eradication of citrus canker in Dade and Broward counties of Florida. Red circles depict areas of 1900-ft radius surrounding a citrus canker-diseased tree, within which citrus trees are defined as exposed.

In December 1998, the epidemiology study of citrus canker spread under urban conditions (see Epidemiology section above) was reviewed by a group of scientists and regulatory officials. The consensus was: (i) the 125-ft (38-m) radius used to define exposure was inadequate to suppress the continued spread of canker; and (ii) although disease spread was detected up to 58850 ft (17942 m), the majority of new canker infections occurred within about 1900 ft (579 m) of known source trees (27). As a result, a new regulation -- the "1900-ft rule" -- was established in January 2000 and put in practice in March 2000, requiring the removal and destruction of diseased citrus trees and of all citrus trees within a 1900-ft radius of a diseased tree (27,30,71) (Fig. 46). The 1900-ft rule was challenged in court but upheld and presently serves as the operational basis of the citrus canker eradication program. Each circle of 1900 ft radius represents 0.41 square miles (1.06 km^2) (Fig. 2). The implementation of the 1900-ft rule results in removal of the majority of dooryard citrus within infected areas.

Sentinel Tree Survey. Removal of diseased and exposed trees is one part of the eradication program. Detection of new infections is another. Citrus canker has continued to spread northward along the east coast of Florida toward the Indian River citrus production area of St. Lucie and Martin counties, which consists mainly of highly susceptible plantings of grapefruit. A second important outcome of the canker epidemiology study is the adoption of sentinel tree survey method to detect new residential infections outside quarantine areas very early and efficiently. Arrays of about 144 existing dooryard trees of susceptible cultivars in a 12-by-12 arrangement [trees approximately 134 m (440 ft) apart] covering each square mile are used as an early-warning system for new canker outbreaks (Fig. 47). Beginning in June of 2000, a 25 km (15 mile) wide by 33 km (20 mile) long sentinel-tree area in Palm Beach County, north of the Miami outbreak area, was visually surveyed on a repeated 30-day rotation (27). As a result, several new outbreaks of canker were detected in Palm Beach County, (Fig. 48) and subsequently in Orange County (Orlando), Brevard County (Cocoa), and Monroe County (Big Pine Key). Detection of these new outbreaks of canker resulted in removal and destruction of diseased and exposed trees under the 579-m (1900-ft) rule. The success of the sentinel tree grid system in detecting citrus canker early in new areas has prompted its expanded use throughout Florida and resulted in further finds in Palm Beach and Brevard counties in early stages of disease development.

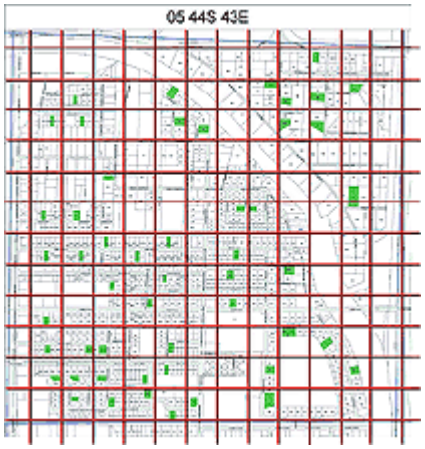

Fig. 47. Example of implementation of the sentinel tree survey method. The grid depicted by the red lines divides the one-square-mile into 144 subsections. Where possible, within each subsection a legal property is selected on which one or more citrus trees highly susceptible to citrus canker are located (depicted in green). The result is an irregular array of trees that can be resurveyed for new canker infections on a 30-day rotation.

Fig. 48. Map of portion of residential Palm Beach County where the sentinel tree survey method is used to search for citrus canker in dooryards. Yellow squares are one-square-mile sections that have been surveyed. Pink squares are sections remaining to be surveyed during the depicted sweep of the area. Red marks indicate properties with citrus canker-infected trees discovered by survey method.

Research and Public Policy. Following the January 2000 implementation of the 1900-ft regulation, approximately 793 square miles (2,054 km^2) of the Miami metropolitan area in Dade and Broward counties would be affected and an estimated 700,000 dooryard trees were to be removed from urban areas in Dade and Broward counties within the next year (27) (Fig. 46). The reaction of residents whose trees have been or will be removed ranges from general acceptance to strong resistance in the form of litigation and occasionally personal threats to eradication program personnel and administrators (27). As a result, several legal actions were taken by homeowners to halt the eradication program. Homeowners felt that current regulatory actions taken protected the citrus industry at too high a cost to residential citrus tree owners. In November 2000, an injunction was placed on the eradication program such that only trees actually showing disease symptoms could be removed. Only 60,000 to 80,000 of the 700,000 infected and exposed trees to be removed remained before the court injunction stopped the program.

The significant legal and political decisions that have been made affecting the citrus canker epidemic and its eradication in Florida are summarized in Table 2 (see Appendix). Appeals and challenges by the eradication program to court decisions have met with a few reversals, along with more court delays and continuances. This had effectively halted the eradication program pending an administrative hearing to determine the state's rule-making authority in the creating of the 1900-ft regulation and to challenge the science behind the eradication program. Dismayed by the lack of protection that a ham-strung eradication program affords the commercial citrus industry, commercial producers in conjunction with the Florida Department of Agriculture and Consumer Services, crafted legislation to codify the 1900-ft rule into state statutes (i.e., state law). On March 15 and 16, 2002, the legislation passed the Florida House of Representatives and Senate, respectively. On March 18, 2002, Governor Jeb Bush signed the bill into Florida law paving the way for the eradication program to begin anew after an 18 month hiatus. Meanwhile, south Florida residents have continued their opposition and some south Florida

counties have challenged the new law, its constitutionality, and the science behind it.

Because of the highly charged political and social atmosphere surrounding citrus canker eradication, researchers working on the disease have found themselves at odds with various groups through time. In the mid 1980s, an entirely new Xanthomonas disease of citrus was discovered causing canker-like symptoms on nursery stock in Central Florida (68). The disease was tentatively identified as a new strain of citrus canker. Because of the urgency of protecting the citrus industry from the universally perceived threat, the new disease was dealt with according to long-standing policy for canker in Florida. In spite of considerable regulatory restraints on any field work with the new pathogen and the disease it caused, the emerging research findings revealed that the new disease was not serious enough to warrant the aggressive actions spelled out in the established citrus canker regulatory policy. The transition phase toward deregulation of the new disease, which has since become known as citrus bacterial spot (pathogen = *Xanthomonas axonopodis* pv. *citrumelo*), was sometimes awkward. Researchers found themselves in the uncomfortable position of providing evidence in the courts that regulatory policies were excessive while regulatory agencies charged with controlling the disease slowly and cautiously adopted less aggressive measures. Eventually, as confidence in emerging research findings was established, the situation developed into a joint collaboration in which specific research was actually requested by regulators to aid in regulatory decision-making. More recently, several research projects on citrus canker itself have indicated that regulatory measures needed to be strengthened and/or expanded to contain canker disease increase and spread. This resulted in the need for more extensive and costly sanitation measures and tree removal in commercial and residential citrus.

Science-based regulatory decisions were unpopular with a few segments of the citrus industry that were hard hit or perceived their livelihood to be in jeopardy. Greatly expanded eradication efforts based on research findings on citrus canker spread in urban settings in south Florida has resulted in removal of thousands of additional trees from commercial and residential areas. The majority of commercial growers and homeowners eventually understand this need to remove a much greater distance of exposed trees in an attempt to halt the epidemic. However, severe opposition was and continues to be voiced by a single south Florida grower, and a small segment of homeowners and municipalities who valued their citrus trees more highly than the needs of the citrus industry in general.

As in many situations where people disagree, legal proceedings commence to resolve the disputes. Much to the chagrin of researchers, not all segments of the population are equally or immediately accepting of the scientific research behind the eradication program. This places the research community in the difficult position of testifying in a court of law in which legal council for the eradication program is supportive of the science while legal council for the plaintiffs attempts to discredit the scientific findings. Needless to say, scientists are usually most comfortable explaining and defending their work when questioned by other scientists in their field who are generally simply seeking justification and understanding and are qualified to assess them. However, researchers are not well trained to explain their research in the legal arena to non-scientists untrained in the discipline and against adversarial legal council whose job it is to discredit them. Harsh as it may seem, neither the general populace nor the affected municipalities truly have the qualifications to judge the science they attempt to discredit, especially when motivated solely by an aversion to the implications and regulatory policies that result. But as a society, it makes good sense to rely upon the most highly trained and experienced experts to generate appropriate research and interpret the results. Unfortunately, such legal challenges to scientific research are becoming more commonplace. The actual dispute is not so much about the validity of the science but how the policy that is fashioned from the science is developed and implemented.

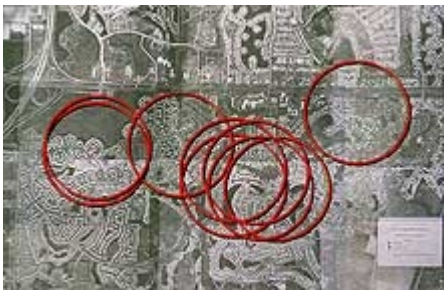

Fig. 49. Aerial map of residential area in Hillsborough County subjected to citrus canker eradication. Circles represent overlapping areas whose center is an infected citrus tree from which a 1900-ft radius is set. Following tree removal the area remained free of citrus canker for two years and has been released from quarantine.

The future. At the time of this writing, the outcome of all the legal positions and maneuvers on the eradication program is uncertain. However, it is certain that the disease will continue to increase and spread regardless of human deliberations (Fig. 3) and it must be recognized that indecision constitutes a choice. Continued delays work against eradication. Complete eradication of an invasive species that has spread to the extent that citrus canker has in Florida is extremely difficult. Some residential areas that have been subjected to the 1900-ft. eradication method have remained free of canker for the prescribed two-year period, have been released from quarantine, and can now replant if they wish (Fig. 49). Some researchers, growers, and residents dispute the concept and feasibility of eradication, and the question thus arises, "Can we live with citrus canker?" Several countries in Southeast Asia, South America and elsewhere have lived with canker for decades; in many cases their industry actually co-evolved with canker from the start. But in all cases the industry is considerably altered and production costs increased. For example, some cultivars are too susceptible and are no longer commercially feasible to grow, and national and international markets are lost due to quarantines and embargos (61,62). The obvious implication is that failure to attempt eradication will result in severe political and economic penalties for Florida's citrus industry. Unfortunately, even if eradication is achieved, there is a high probability for reintroduction of *Xac* in the future, unless better safeguarding takes place. Some of these safeguarding initiatives are already underway, such as the permanent implementation of the sentinel survey. At least three separate introductions of *Xac* have been discovered in Florida since 1986, indicating that introduction of the disease is a fairly regular occurrence. If eradication is achieved, greater emphasis on the laws pertaining to plant introduction, better stringency at customs, plus surveillance programs such as the sentinel tree survey will need to be maintained indefinitely in order to realize the benefits of eradication for the future and maintain a canker-free status for all of Florida's citrus, both private and commercial.

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Appendix

Table 2. Significant Events Relating to Spread of Canker in Florida, 1995 to present

Date	Event	Significance
1995	Canker discovered in suburban Miami in late summer.	Canker reappears in Florida after an apparent 3-year absence. Disease discovered while trapping for fruit flies. Pathogen is genetically different from that present in 1986-92 in Manatee County area.
	Initial 14-mi^2 zone of canker infected trees grows to a 100-mi^2 quarantine zone by year's end.	Only dooryard citrus has canker at this time. No commercial citrus infected. Infected trees are removed, and exposed trees within 125 ft are pruned to brown wood.
1996	Tornado passes through infected zone resulting in increase from 14 to 44 mi^2 infected.	Efforts so far have not stopped disease spread. A high percentage of the pruned trees have subsequently become infected.
	Using biometric survey methods, canker is found outside the quarantine zone twice during the year.	Efficient surveys must be conducted inside and outside quarantine area to delimit disease and are essential to success of program.
	Quarantine zone first expanded to 165 mi^2, then to 265 mi^2.	Each expansion creates an enormous increase in workload and infrastructure.
	Three major tropical weather events occur during the year [Tropical Storms (TS) Bertha and Josephine, and Hurricane Lilli].	Unpredictability of catastrophic weather events underscores the urgency of keeping the number of known diseased trees to a minimum to avoid long distance inoculum dispersal.
Date	**Event**	**Significance**
1997	CCEP decides to remove rather than prune exposed trees within 125 ft of a diseased tree.	Pruning to avoid infection of exposed symptomless trees was a failure. Removal of symptomless trees is very unpopular with public.
	Canker is discovered again in Manatee County in commercial citrus. 45 mi^2 quarantine area established resulting from TS Josephine.	First involvement of commercial citrus since 1995. Origin of disease is likely holdover from the 1986-94 eradication effort in this area. Need to continue survey after eradication is reinforced.
	Cost sharing between USDA and FDACS has provided about $9 million so far (about $1.3 million of that federal funds). State receives $17 million in legal settlement from USDA for failure to cost-share as	Funding to undertake timely regulatory action is always problematic.

	contracted in previous eradication program.	
1998	Florida Commissioner of Agriculture Bob Crawford announces a one year moratorium on cutting exposed citrus concurrently with initiation of urban epidemiology study.	Public outcry against cutting exposed citrus in residential areas intensifies. Moratorium allows study to be done in residential areas of Miami-Dade and Broward counties. Only infected trees cut during this moratorium.
	To date, seven commercial groves in Manatee County are discovered with canker.	Disease was active here for about two years before it was discovered, and was spread by cultural activities as well as weather events.
	Two commercial groves in Collier County are found with canker.	Canker now established in the heart of the expanding SW Florida citrus industry from the Miami metro area.
	Two hurricanes (George and Mitch) in the fall create both short and long distance inoculum spread.	Tropical weather is unpredictable, exacerbates disease spread, and complicates eradication.
	Early analysis of epidemiology study data reveals that a much larger than 125-ft exposure radius must be utilized if canker is to be eradicated.	Data is still being gathered and will be further analyzed over the next couple of years. A distance of 1900 ft appears to be sufficient to capture the majority of new canker-infected trees during a 30-day period.
Date	**Event**	**Significance**
1999	Canker spreads into Broward County with a firmly established foothold when discovered in the Coral Springs area.	Movement of plant material out of quarantine zone is circumstantially implicated as the method of dispersal.
	Canker discovered in Hendry County.	Miami genotype is associated by bacterial genotype analysis.
	Canker found in eastern Manatee County commercial grove.	New quarantine area required to encompass outbreak. Miami genotype is associated.
	Hurricanes Floyd and Irene and TS Harvey occur during the late summer and fall season.	Threat of inoculum dispersal and very rapid local disease build-up if infected trees present during storms.
	Four residential outbreaks; two in Hillsborough County and two in Collier County.	Canker eradication is more challenging in small residential parcels. Mixed Miami and Manatee pathogen genotypes in one Hillsborough County site; Miami genotype of the pathogen is present in all other areas.
	Crop insurance expanded by federal government. $16 million appropriated for canker program by U.S. Congress.	Crop insurance provides inducement for cooperation among commercial growers hit with canker.

2000	Canker discovered in commercial lime groves of Dade County.	Significant expansion of disease into the main lime production area of the U.S. results from Hurricane Irene.
	Emergency declaration by Governor Jeb Bush.	Continued spread of canker in spite of eradication efforts prompts declaration. Faster rule making-process now in place
	FL Agriculture Commissioner announces an all-out plan to eradicate canker over the next year by cutting all known infected and exposed trees to 1900-ft radius, step up survey efforts, continue tree canopy replacement program in residential areas.	Acknowledgement that previous efforts were falling short of the statutory goal of canker eradication.
	Routine application of 1900-ft exposure radius begins.	Removal of exposed trees to this expanded radius dramatically reduce the number of newly infected trees.
	Univ. of Florida conducts first public opinion poll of Dade County residents concerning the eradication campaign.	Level of support for program is just under 80%.
	Canker spreads into residential areas of Palm Beach County.	Northward spread bringing canker closer to Indian River citrus production area where majority of highly susceptible grapefruit is grown.
	Statewide decontamination rules enacted.	Circumstantial evidence that harvesting operations are likely the method for orchard to orchard spread makes this action necessary.
	Wellington strain of canker (Aw) discovered in Palm Beach County.	Unusual strain with host range restricted mainly to Key lime. Apparently in area for several years prior to discovery.
	By midyear, total trees removed in Dade, Broward, and Palm Beach counties is 199,458; 27,856; and 196 respectively.	Southeast residential area poses the greatest obstacle to eradication. Disease continues to spread from these counties. Because of magnitude of eradication program, social and legal obstacles to eradication develop.
	Circuit Court Judge issues injunction on cutting exposed trees. At the same time, additional litigation in other courts is dismissed in favor of the eradication program. Stalls eradication program.	The court wants reviews of possible cures for canker, necessity of cutting exposed trees, homeowner compensation, and compliance with rule-making process.
	To comply with circuit judge	Community liaisons are placed in

	request, CCEP institutes improvements to public relations policy.	county administration offices and city halls, public hearings are held to allow more citizen input, telephone help-line staff is doubled, and a troubleshooting team is established to handle citizen complaints promptly.
	Sentinel survey program is initiated. Program is outgrowth of urban epidemiology study.	Survey method is applied statewide to detect citrus canker and eventually other exotic pests and diseases earlier.
Date	**Event**	**Significance**
2001	First publication of urban epidemiology study is released (27).	Publication validates the need for a greater radius for removal of exposed trees in order to effectively eradicate citrus canker.
	Two more public opinion polls conducted in Dade and Broward County areas to measure support for program.	Polls show support level for program is now at 70-75%.
	Program costs to date are about $200 million.	Projecting costs is extremely difficult because success depends largely on the outcome of litigation, which itself can be very costly.
	Florida Citrus Mutual releases a study that estimates the cost of living with citrus canker in Florida would be $342 million per year.	The estimates are for commercial disease management costs and crop losses only; no estimate of impact to residential citrus. Fosters additional citrus industry support for eradication.
	Circuit court still deliberating, injunction against cutting exposed trees continues.	Legal delays allow continued spread of disease. Judge reviews adequacy of homeowner compensation for lost trees.
	Circuit court still has not rendered a decision, and is now deliberating whether a class action suit is warranted.	Continued deliberation results in more citrus canker infection, thus increasing the eventual costs of eradication.
	Appellate court overrules Circuit Court Judge who placed injunction on cutting of exposed trees.	Mixed signals from the judiciary make it difficult for the program to go forward with confidence.
	Appellate court rules that CCEP does not need search warrant to inspect trees on private property.	Plaintiffs challenge authority of regulatory agency access to private property in performing eradication.
	Total citrus trees removed reaches two million.	1.4 million commercial and 0.6 million residential citrus trees removed.
	Florida Administrative Law Judge rules that emergency eradication rule as written is too vague in the exercise of	State's rule-making process is declared invalid, rewrite of emergency rule to correct problem is also challenged.

	risk assessment powers.	
	Lawsuit challenges the government's authority to enter private property without a search warrant.	Objections to program shift from disputing the science to challenging the constitutionality of inspection process.
	By year's end, canker was found in ten separate commercial incidents; five in residential areas outside of southeast Florida, with finds almost daily in Miami-Dade and Broward counties. Martin and De Soto counties added, for total of nine infested counties.	Canker eradication successful everywhere except the residential Miami-Dade, Broward, and Palm Beach counties, which continue to act as the predominant inoculum source.
2002	Administrative hearing postponed for third time.	Basis for continuance is the complexity of the case and the plaintiffs' insistence that they need more time to prepare.
	Circuit Court Judge grants class action status to homeowners who contend they have received inadequate compensation for trees removed by the program.	Judge effectively reversed all previous adjudications of this matter by granting class action status to an estimated 100,000 residents of Miami-Dade and Broward counties.
	Commercial citrus industry frustrated by legally stalled eradication program in Southeast Florida counties.	Citrus industry lobbies Florida legislators to introduce new eradication bill.
	Florida Legislature circumvents the rule-making process by overwhelmingly voting to place the 1900-ft exposure radius into statute (law) rather than rule.	Legal challenge to rule-making authority and process is no longer an issue. The mandate to eradicate canker and 1900-ft method are given a statutory basis. Injunction on cutting exposed trees overruled by legislature.
	Canker discovered in residential Brevard County	Ten counties infested since 1996, but Hillsborough County is removed from quarantine after submitting to 1900-ft rule and staying citrus canker-free for 2 years.
	Second publication relating to the epidemiology study is released (30).	The scientific basis for the 1900-ft statute is further validated by data and analyses presented in this paper.
	Broward County and associated municipalities challenge constitutionality of new legislation.	Statue allows county-wide search warrants for inspection and removal of infected trees. Legal challenges shift further from scientific issues to homeowner rights.